Journal of Medical Virology 59:232–238 (1999)

High Prevalence of Human Papillomavirus Type 58 in Chinese Women With Cervical Cancer and Precancerous Lesions

Paul K.S. Chan,[1]* Wai-Hon Li,[2] May Y.M. Chan,[2] Wei-Ling Ma,[2] Jo L.K. Cheung,[1] and Augustine F. Cheng[1]

[1]Department of Microbiology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong
[2]Department of Obstetrics and Gynaecology, Queen Elizabeth Hospital, Kowloon, Hong Kong

The prevalence of human papillomavirus (HPV) among 332 Hong Kong Chinese women with abnormal Papanicolaou smears were determined by polymerase chain reaction and restriction fragment length polymorphism analysis. The overall HPV positive rate was 44.3% with 18.6% (16/86) for normal/inflamed cervices, 36.4% (32/88) for condyloma, 64.7% (33/51) for cervical intraepithelial neoplasia grade 1 (CIN 1), 37.9% (11/29) for CIN 2, 68.3 (41/60) for CIN 3, and 77.8% (14/18) for carcinoma. Double HPV infection was detected in 17 of the 147 positive samples, with a significantly higher proportion in patients with normal or inflamed cervices than those with CIN or carcinoma (31.3% vs 10.5%, $P = .029$). The six most commonly identified genotypes were HPV 16 (33.3%), HPV 58 (23.8%), HPV 11, 18, 31 (8.8% each), and HPV 33 (6.8%). The worldwide uncommon genotype HPV 58 was found to be the second most common genotype detected in patients with cervical carcinoma (6 of 18 patients). HPV 58 infection showed a significant association with CIN/carcinoma (odds ratio [OR] = 3.98; 95% confidence interval [CI] = 1.22–14.35) and a significant trend of increase in prevalence with increasing severity of cervical lesion ($\chi^2 = 5.84$; $P = .016$). Among Hong Kong Chinese women with abnormal cervical cytology, the detection of HPV 58 carried a positive predictive value of 68.6% for a cervical lesion of CIN 1 or higher severity. The high prevalence of HPV 58 among Chinese women, particularly in patients with carcinoma, has an implication on the design of HPV detection methods and the development of vaccines. ***J. Med. Virol. 59:232–238, 1999.***

KEY WORDS: HPV prevalence; HPV genotypes; polymerase chain reaction; restriction fragment length polymorphism; multiple infection; cervical intraepithelial neoplasia

INTRODUCTION

Papillomaviruses are a heterogeneous group of DNA viruses, which occur predominantly in squamous epithelium causing hyperplastic, papillomatous, and verrucous squamous epithelial lesions in humans and in a wide range of animals. To date, more than 85 human papillomavirus (HPV) types have been identified, of which at least 28 types have been found in female genital tract infections [Munoz and Bosch 1992; De Villiers 1994; Van Ranst et al., 1996]. Strong evidence accumulated from epidemiological surveys, clinical observations, and molecular biological studies has implicated an etiologic role for HPV infection in the development of cervical intraepithelial neoplasia (CIN) and cervical cancer [Herrington, 1994, 1995]. HPVs have been categorized into "high-risk" (HPVs 16, 18, 45, and 56), "intermediate-risk" (HPVs 31, 33, 35 51, 52, and 58), and "low-risk" (HPVs 6, 11, 42, 43, and 44) groups based on their relative risks for the occurrence of a high-grade cervical lesion and an invasive cancer [Lorincz et al., 1992].

Although cervical cancer is relatively common in the People's Republic of China [Pao et al., 1993], little research has been published in the West regarding the prevalence of HPV infection in Chinese women. Studies from Central and South America, Europe, Africa, and Southeast Asia have consistently showed a high prevalence (75–100%) of HPV infection among women with invasive cervical cancer [Bosch et al., 1995]. In contrast, Pao et al. [1994] found evidence of HPV in-

Grant sponsor: Queen Elizabeth Hospital; Grant number: R95-2-3.

*Correspondence to: Dr. Paul K. S. Chan, Department of Microbiology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong. E-mail: paulkschan@cuhk.edu.hk

Accepted 17 December 1998

fection in only 57% of women with invasive cancer living in Shanxi, China, where the incidence of cervical cancer is extremely high (1,026 per 100,000). Another unique feature of the epidemiology of HPV infection in Chinese women is the high prevalence of HPV 52 and HPV 58 infections (15 of 35 cases) among women with cervical cancer living in Shanghai, China [Huang et al., 1997]. This finding is in contrast to a recent report from an international study, which showed that HPV 16 was most predominant (51%) in patients with cervical cancer in America, Europe, Africa, and Southeast Asia [Bosch et al., 1995].

Geographic clustering of some less common HPV types has been documented. HPV 45 was apparent in western Africa, whereas HPV 39 and HPV 59 were almost exclusively confined to Central and South America [Bosch et al., 1995]. Understanding the distribution of cancer-associated HPV types has important implications for the development of vaccines for the prevention of cervical cancer. To elucidate further the epidemiology of HPV infection in the Chinese population, we sought to determine the type-specific prevalence of HPV infection in women with cervical lesions living in the Hong Kong Special Administrative Region, People's Republic of China.

MATERIALS AND METHODS

Study Population

During the 11-month study period, Chinese women who were referred to the colposcopy clinic at the Queen Elizabeth Hospital for further management following abnormal Papanicolaou smears were included. Women who were pregnant, immunocompromised, or who had a previous history of condyloma, premalignant, or malignant disease of the cervix were excluded. A written consent was obtained from all participants and the study was approved by the local ethics committee. In Hong Kong, cervical cancer accounts for 2.7% of all newly diagnosed cancers and 1.5% of all cancer deaths, and the age standardized incidence rate of cervical cancer is 14.1 per 100,000 females with a mean age at diagnosis of 59 years old [Hong Kong Cancer Registry, 1992].

Colposcopy was undertaken for all women. CIN classification and diagnosis of carcinoma were based on histologic findings.

Specimen Collection

Cervical exfoliated cells were collected by an Ayre spatula, which was then agitated in 10 ml of phosphate-buffered saline (PBS). Cells were pelleted by centrifugation and resuspended in 1 ml of PBS, and stored at −70°C until further analyses. Cell suspensions were heated at 95°C for 10 min before subjection to polymerase chain reaction (PCR). The quality of sample was assessed by PCR using β-globin primers PC03 and PC04 [Saiki et al., 1986] to confirm the presence of an adequate preparation of DNA and that nonspecific inhibitors were absent.

HPV Detection

HPV DNA was detected by a "hot-start" PCR using degenerate HPV-consensus primers MY09, 5′-CGTCCMARRGGAWACTGATC-3′ and MY11, 5′-GCMCAGGGWCATAAYAATGG-3′ (M = A or C; R = A or G; W = A or T; Y = C or T) [Ting and Manos, 1990]. These primers are capable of amplifying a 450-bp DNA fragment from the L1 open reading frame (ORF) of at least 40 genital HPV types [Bauer et al., 1991; Bernard et al., 1994]. The PCR was performed in a 100-μl reaction mix containing 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each deoxynucleoside triphosphate, 0.5 μM of each primer, 2 units of *Taq* polymerase (Pharmacia Biotech, Sweden) and 10 μl of cervical cell suspension. The thermal cycling conditions were: (a) denaturation at 95°C for 5 min; (b) hold at 85°C for the addition of 20 μl of *Taq* polymerase at a concentration of 0.1 unit/μl; (c) 40 cycles of 95°C for 30 sec, 55°C for 1 min, and 72°C for 1.5 min; and (d) final extension at 72°C for 8 min. Amplicons were electrophoresed on a 2% agarose gel and stained with ethidium bromide to detect the consensus PCR product of genital HPVs.

All PCRs were performed under conditions designed to minimize contamination [Kwok and Higuchi, 1989]. A negative control containing all PCR reagents except DNA template was added following every fifth specimen to monitor for contamination. Four positive controls consisting of approximately 500 and 100 copies of full-length HPV 16 and HPV 6 genome, respectively, were included in each PCR run to monitor for sensitivity. Serial dilutions of recombinant plasmids containing full-length HPV 6, 11, 16, and 18 genomes were used to estimate the analytic sensitivity of the PCR. The amplification reactions allowed consistent and reproducible detection of at least 100 copies of these HPV genomes.

HPV Typing by Restriction Fragment Length Polymorphism

HPV types were determined by restriction enzyme digestion of the consensus PCR product [Bernard et al., 1994]. Five microliters of unpurified PCR product was digested by restriction endonucleases *Rsa* I and *Dde* I separately in a 10-μl reaction mix containing the enzyme at a concentration of 0.1 unit/μl. After an overnight incubation at 37°C, the reaction was terminated with stopping buffer. Digested fragments were electrophoresed on a 3% agarose gel (MetaPhor™ Agarose, FMC BioProducts, Denmark).

Statistical Analysis

Association between HPV infections and cervical lesions were assessed by calculating the odds ratios and adjusted for possible confounding factor by using Mantel–Haenszel χ^2 test. The linear trend of proportion of HPV infections among various categories of cervical lesions and age groups were assessed by χ^2 test for trend. The difference in proportion of single and double HPV infections among women with normal/inflamed cervi-

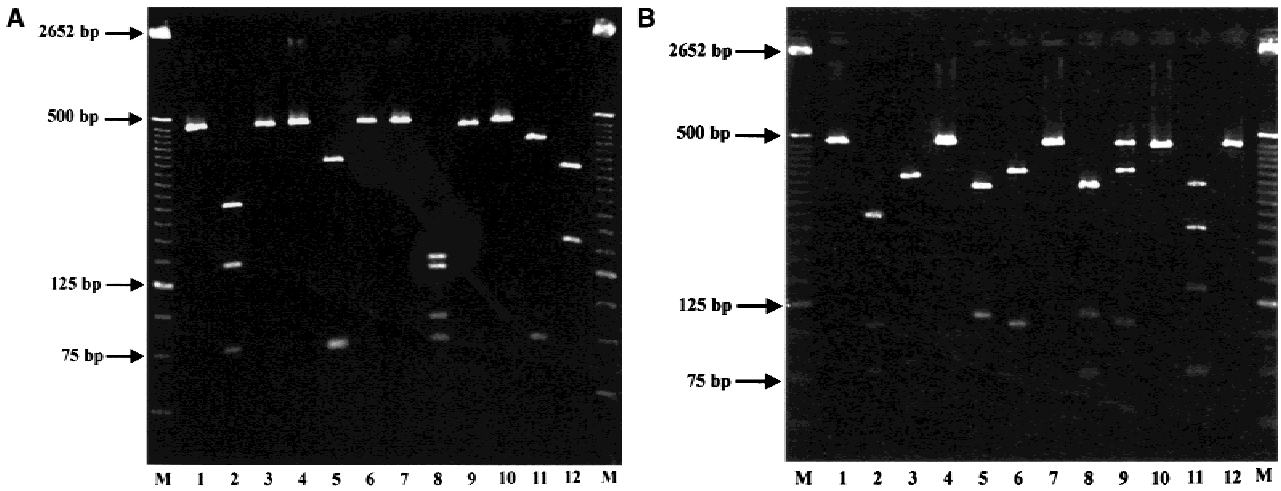

Fig. 1. Restriction fragment length polymorphisms of the six single types and the two double types of human papillomavirus (HPV) infections most commonly detected in Chinese women with cervical lesions. **(A)** Lane M, 25-bp DNA ladder markers; lanes 1–12: HPV 11 not restricted; restricted with *Rsa* I; restricted with *Dde* I; HPV 16 not restricted; restricted with *Rsa* I; restricted with *Dde* I; HPV 18 not restricted; restricted with *Rsa* I; restricted with *Dde* I; HPV 31 not restricted; restricted with *Rsa* I; restricted with *Dde* I. **(B)** Lane M, 25-bp DNA ladder markers; lanes 1–12: HPV 33 not restricted; restricted with *Rsa* I; restricted with *Dde* I; HPV 58 not restricted; restricted with *Rsa* I; restricted with *Dde* I; HPV 16 and 58 not restricted; restricted with *Rsa* I; restricted with *Dde* I; HPV 11 and 16 not restricted; restricted with *Rsa* I; restricted with *Dde* I.

ces and CIN/carcinoma was assessed by Fisher's exact test. All analyses were two-tailed and $P < .05$ at 95% confidence limit was regarded as significant.

RESULTS

A total of 332 Chinese women who fulfilled the inclusion criteria and who had cervical samples with an adequate quality of DNA were included in the study. Their ages ranged from 16 to 78 years (mean, 40 years). Eighty-six had either normal or inflamed cervices, 88 had condyloma, 51 had cervical intraepithelial neoplasia 1 (CIN 1), 29 had CIN 2, 60 had CIN 3, 16 had squamous cell carcinoma, and 2 had adenocarcinoma in situ.

Overall, 44.3% (147/332) of the cervical samples were

TABLE I. Prevalence of Human Papillomavirus (HPV) Infections in 332 Chinese Women With Various Grades of Cervical Lesions

	Number (%) of women with						
HPV infection	Normal/inflamed cervix	Condyloma	CIN[a] 1	CIN 2	CIN 3	Carcinoma[b]	Total
Positive	16 (18.6)	32 (36.4)	33 (64.7)	11 (37.9)	41 (68.3)	14 (77.8)	147 (44.3)
Single type	11 (12.8)	25 (28.4)	31 (60.8)	9 (31.0)	34 (56.7)	12 (66.7)	122 (36.7)
Double types	5 (5.8)	3 (3.4)	2 (3.9)	2 (6.9)	3 (5.0)	2 (11.1)	17 (5.1)
Types[c]							
HPV 6	1 (1.2)	1 (1.1)	1 (2.0)	0	0	0	3 (0.9)
HPV 11	4 (4.7)	7 (8.0)	0	1 (3.4)	1 (1.7)	0	13 (3.9)
HPV 16	4 (4.7)	3 (3.4)	11 (21.6)	4 (13.8)	19 (31.7)	8 (44.4)	49 (14.8)
HPV 18	1 (1.2)	2 (2.3)	5 (9.8)	2 (6.9)	2 (3.3)	1 (5.6)	13 (3.9)
HPV 31	3 (3.5)	2 (2.3)	4 (7.8)	1 (3.4)	2 (3.3)	1 (5.6)	13 (3.9)
HPV 33	0	3 (3.4)	1 (2.0)	0	6 (10.0)	0	10 (3.0)
HPV 39	0	1 (1.1)	0	0	1 (1.7)	0	2 (0.6)
HPV 52	1 (1.2)	1 (1.1)	3 (5.9)	0	1 (1.7)	0	6 (1.8)
HPV 53	1 (1.2)	3 (3.4)	0	1 (3.4)	1 (1.7)	0	6 (1.8)
HPV 56	0	0	1 (2.0)	0	0	0	1 (0.3)
HPV 58	4 (4.7)	7 (8.0)	8 (15.7)	4 (13.8)	6 (10.0)	6 (33.3)	35 (10.5)
HPV 61	2 (2.3)	1 (1.1)	0	0	1 (1.7)	0	4 (1.2)
HPV 68	0	0	1 (2.0)	0	0	0	1 (0.3)
Others[d]	0	4 (4.5)	0	0	4 (6.7)	0	8 (2.4)
Negative	70 (81.4)	56 (63.6)	18 (35.3)	18 (62.1)	19 (31.7)	4 (22.2)	185 (55.7)
Total	86	88	51	29	60	18	332

[a]CIN, cervical intraepithelial neoplasia.
[b]Carcinoma, 16 cases of invasive squamous cell carcinoma and 2 cases of adenocarcinoma in situ.
[c]Regardless of single-type or double infection.
[d]Include all uncharacterized HPV types.

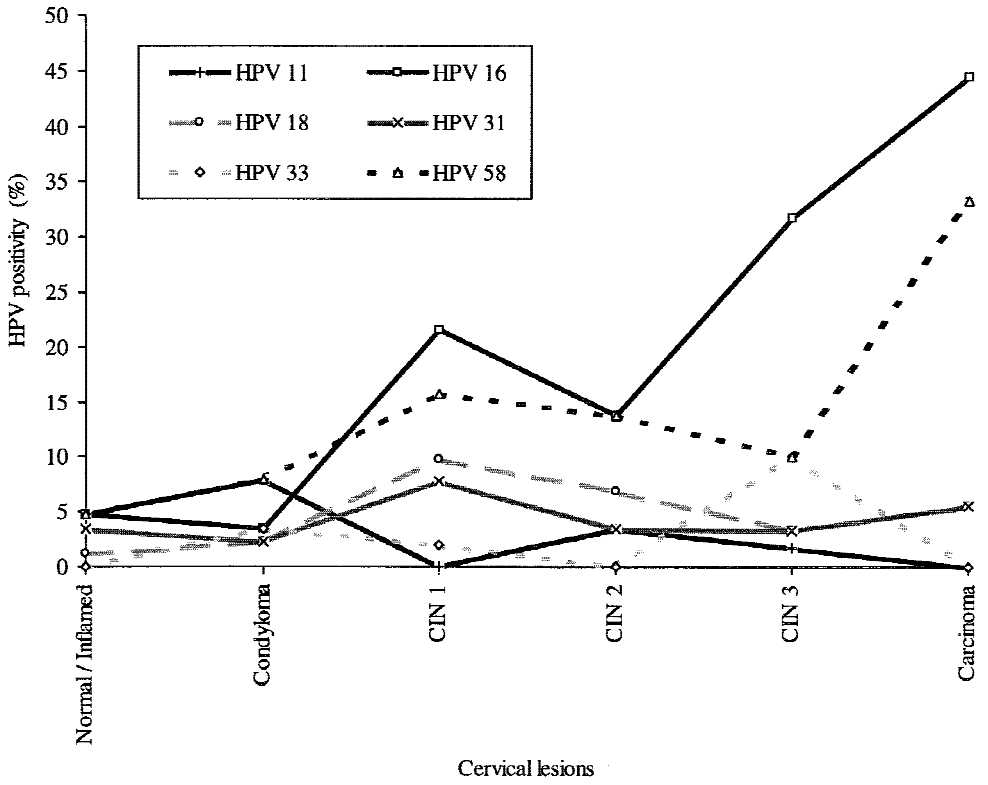

Fig. 2. Prevalence of the six most common human papillomavirus (HPV) types in 332 Chinese women by various grades of cervical lesions.

positive for HPV DNA, with a prevalence of 18.6% for normal or inflamed cervices, 36.4% for condyloma, 64.7% for CIN 1, 37.9% for CIN 2, 68.3% for CIN 3, and 77.8% for carcinoma.

The results of typing by restriction fragment length polymorphism analysis are shown in Figure 1. Overall, the types identified most frequently were HPV 16 (33.3%), HPV 58 (23.8%), HPV 11, 18, 31 (8.8% each), and HPV 33 (6.8%) (Table I). The prevalence of these six most common types with respect to various grades of cervical lesions is shown in Figure 2. HPV 16 and HPV 58 were the two most frequent types identified from patients with cervical carcinoma. Women with HPV 16 or HPV 58 infection showed a statistically significant increased risk for CIN/carcinoma (odds ratio [OR] = 7.42, 95% confidence interval [CI] = 2.42–25.41 for HPV 16; OR = 3.67, 95% CI = 1.15–12.98 for HPV 58). The association between HPV 58 and cervical lesions was still significant after adjusting for HPV 16 DNA positivity (OR = 3.98, 95% CI = 1.22–14.35) (Table II). For both types, there was a significant trend of increase in prevalence with increasing severity of cervical lesion (χ^2 = 22.53, $P < .001$ for HPV 16; χ^2 = 5.84, P = .016 for HPV 58). HPV 11 was the most frequent type identified from patients with normal/inflamed cervices or condyloma. However, a significant trend of decrease in the prevalence of HPV 11, a low-risk type, with increasing severity of cervical lesion was not observed. No significant trend of distribution of HPV 18, 31, and 33 for different grades of cervical lesions was observed. Among Hong Kong Chinese women with abnormal cytology results, the detection of HPV 16 and HPV 58 carried positive predictive values of 85.7% and 68.6%, respectively, for a cervical lesion of CIN 1 or higher severity, whereas the positive predictive value of HPV 11 for a normal/inflamed cervix or condyloma was 84.6%.

Among the HPV-positive patients, 83.0% (122/147) had a single HPV type detected, and 11.6% (17/147 cases) were double infections. In 8 patients, an amplicon of expected size (~450 bp) was detected indicating the presence of genital HPV DNA. However, a definitive type could not be identified based on their restriction fragment length patterns. Among HPV-positive patients with identifiable types, the proportion of double HPV infection was significantly (P = .029) higher in patients with normal or inflamed cervices (31.3%) than those with CIN 1 or higher severity (mean 10.5%, range 6.1–18.2%) (Table I). When the associations between cervical lesions (CIN or carcinoma) and the two categories of HPV infections (single and double) were compared, single-type HPV infection was associated with a significantly higher risk for CIN or carcinoma (OR = 4.34, 95% CI = 1.03–18.02). Of all the double infections identified, HPV 16 and HPV 58 was the most frequently detected combination (5 of 17 patients). The type-specific distribution of double infections are shown in Table III.

TABLE II. Distribution of HPV 16 and HPV 58 Infections in Chinese Women With Normal Cervices and Cervical Lesions

	Normal/inflamed cervix		Cervical neoplasia/carcinoma	
	HPV 58-positive	HPV 58-negative	HPV 58-positive	HPV 58-negative
HPV 16-positive	1	3	4	38
HPV16-negative	3	79	20	96

HPV, human papillomavirus; OR, odds ratio; CI, confidence interval.
HPV 58: OR = 3.67, 95% CI = 1.15–12.98; HPV 16: OR = 7.42, 95% CI = 2.42–25.41; HPV 58 adjusted for HPV 16: OR = 3.98, 95% CI = 1.22–14.35.

When analysed by age groups of <30, 30–39, 40–49, 50–59, and >59 years, the proportion of HPV-positive patients showed a significant linear trend of decrease with increasing age ($\chi^2 = 5.04$, $P = .02$) (Fig. 3). However, no observable trend of distribution of double HPV infection and the two most prevalent types, HPV 16 and HPV 58, with respect to age groups was detected.

DISCUSSION

In the present study, based on a single-step PCR, the prevalence rates of HPV infection among Hong Kong Chinese women with normal/inflamed cervices, CIN 1–3 and carcinoma were found to be 18.6%, 60.7%, and 77.8%, respectively. These prevalence rates are within the reported range among the non-Chinese populations [Lauricella-Lefebvre et al., 1992; Lorincz et al., 1992; Lungu et al., 1992; Vandenvelde et al., 1992]. We found that 11.6% of HPV infections were double infections. The prevalence of multiple HPV infection has varied greatly in different studies and comparing results generated by different methods is difficult [Burmer et al., 1990; Gravitt et al., 1991; Lorincz et al., 1992; Chang et al., 1997]. Genotyping based on restriction fragment length polymorphisms may have difficulties in identifying multiple infection of more than two types, which is likely to exist in our study population. On the other hand, our approach of using consensus PCR followed by restriction fragment length polymorphism analysis is likely to detect double infection only when both types exist in comparable amounts. Therefore, this finding may be more indicative of a significant double infection. We found that a significantly higher proportion of double infections was detected in women with normal or inflamed cervices than those with CIN 1 or higher severity. When compared with single-type infection, double infection showed a significantly lower risk for CIN or carcinoma. These observations may have an implication on the oncogenesis of cervical neoplasia. A widely accepted model of progression of an HPV-infected cell to neoplasia requires the integration of the HPV genome and at the same time disruption of the E2 ORF, resulting in an unregulated production of E6 and E7 oncoproteins. If an E2-encoded protein of one HPV type can produce suppressive effect on E6 and E7 ORFs of other HPV types, a multiple infection may carry a lower risk for malignant progression than infection with a single high-risk type. The lower risk may be because, according to this hypothesis, for a cervical cell infected with multiple HPV types to progress to malignancy, E2 inactivation events will have to occur in each of the existing types. Otherwise, an alternative oncogenic pathway bypassing the E2 inactivation step is required. Alternatively, the CIN/carcinoma group showed a lower prevalence of double infection may be because they were infected at an earlier point in time than the cytologically normal/inflamed group and so have had more time to clear second types through an immune response. Several studies, however, have shown that the proportion of multiple infection decreases with increasing severity of cervical lesion, which is in line with our observations and with the above hypotheses [Lungu et al., 1992; Chang et al.,

TABLE III. Type-Specific Distribution of Double HPV Infections in Chinese Women With Various Grades of Cervical Lesions

	Number of women with		
HPV infection	Normal/inflamed cervix	Condyloma	Cervical intraepithelial neoplasia/carcinoma
6 + 31	1	0	0
6 + 58	0	1	0
11 + 16	1	0	2
11 + 53	0	1	0
16 + 33	0	0	1
16 + 53	0	0	1
16 + 58	1	0	4
18 + 31	0	0	1
18 + 58	0	1	0
53 + 58	1	0	0
58 + 61	1	0	0

HPV, human papillomavirus.

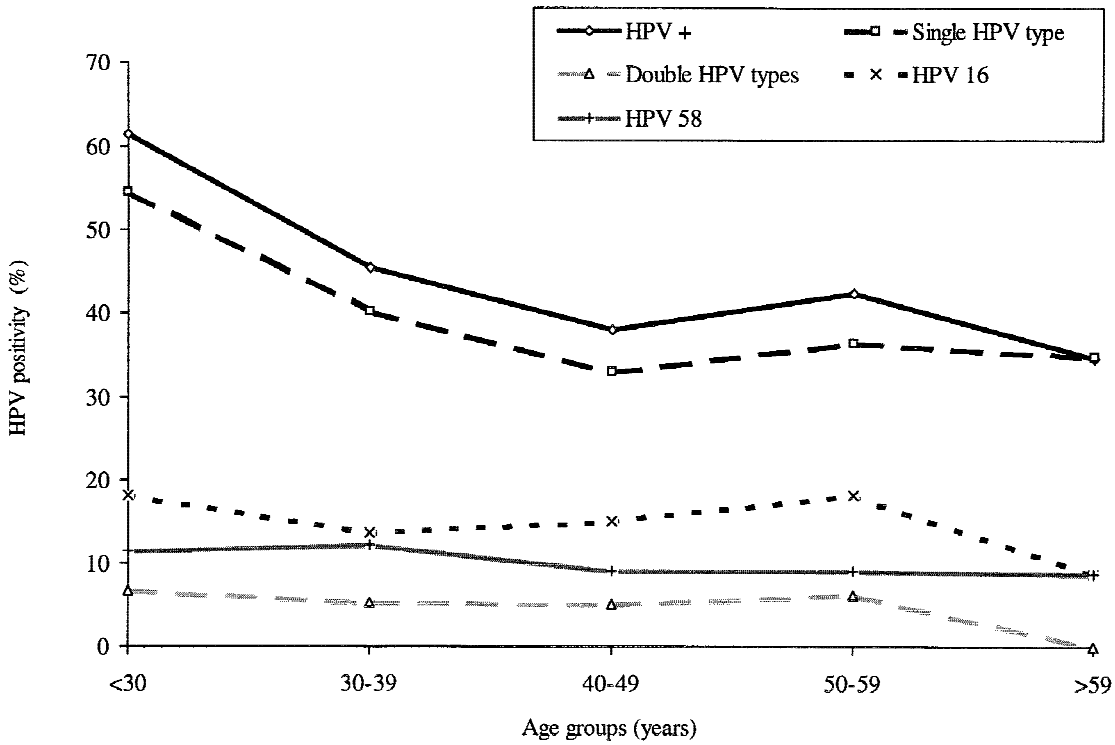

Fig. 3. Prevalence of human papillomavirus (HPV) infections in 332 Chinese women with abnormal cervical cytology by age groups.

1997]. These hypotheses can be tested only by a prospective study with repeated HPV DNA measurements in which women with single and multiple infection are followed over time and their respective incidence of CIN compared.

A further finding of our study is the detection of a relatively high prevalence of HPV 58 infection among women with all grades of cervical lesions. An international study that included cervical cancer specimens from Africa, North and South America, Southeast Asia, and Europe showed only 2% contained HPV 58 [Bosch et al., 1995]. We found, however, that HPV 58 was detected in 33.3% of Hong Kong Chinese women with cervical carcinoma. In women with CIN 1 or higher severity, the prevalence of HPV 58 (15.2%) was second only to HPV 16 (26.6%). As with HPV 16, a significant disease association of HPV 58 was observed. A high prevalence of this worldwide uncommon genotype, HPV 58, has been reported among Chinese women living in Taiwan [Liaw et al., 1995] and Shanghai [Huang et al., 1997]. In these two studies, HPV 52 was also detected at a high frequency comparable to that of HPV 58. However, our results showed that HPV 52 accounted for only 1.8% of the HPV-positive patients. These observations may indicate a variation of HPV epidemiology among Chinese women living in different geographic areas. Apart from the Chinese population, Matsukura and Sugase [1990] also reported a relatively high prevalence of HPV 58 in 4 of 50 Japanese women with cervical carcinoma. It may be worthwhile to elucidate further the epidemiology of HPV 58 with particular reference to Chinese women and women living in Southeast Asia.

Although HPV 16 has been implicated as the most frequent genotype associated with cervical cancer worldwide, our findings and those of Matsukuda and Sugase [1990], Liaw et al. [1995], and Huang et al. [1997] suggest that HPV 58 may also play a significant role in the etiology of cervical cancer, at least, in certain populations. HPV 58 was first cloned in 1990 and was shown to be closely related to HPV 33 [Matsukura and Sugase, 1990]. These viruses, HPVs 16, 31, 33, 35, 52, 58, and 67, are all grouped together under the same branch of an HPV phylogenetic tree, indicating similarities in their pathogenic potential [Bernard et al., 1994].

The findings indicate that although the overall prevalence of HPV among Hong Kong Chinese women is similar to that described in Western countries, the distinct high proportion of HPV 58 infection deserves attention. The fact that a worldwide uncommon genotype, HPV 58, is highly prevalent among Chinese population, particularly in those with cervical cancer, should be considered in the design of HPV detection methods and the development of vaccines for the prevention of cervical cancer.

ACKNOWLEDGMENTS

We thank all the women who participated in this study, Dr. Irene Y. N. Hon for assistance in collection of clinical samples during the initial phase of study.

REFERENCES

Bauer HM, Ting Y, Greer CE, Chambers JC, Tashiro CJ, Chimera J, Reingold A, Manos MM. 1991. Genital human papillomavirus infection in female university students as determined by a PCR-based method. JAMA 265:472–477.

Bernard H-U, Chan S-Y, Manos MM, Ong C-K, Villa LL, Delius H, Peyton CL, Bauer HM, Wheeler CM. 1994. Identification and assessment of known and novel human papillomaviruses by polymerase chain reaction amplification, restriction fragment length polymorphisms, nucleotide sequence, and phylogenetic algorithms. J Infect Dis 170:1077–1085.

Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, Schiffman MH, Moreno V, Kurman R, Shah KV, International Biological Study on Cervical Cancer (IBSCC) Study Group. 1995. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. J Natl Cancer Inst 87:796–802.

Burmer GC, Parker JD, Bates J, East K, Kulander BG. 1990. Comparative analysis of human papillomavirus detection by polymerase chain reaction and Virapap/Viratype kits. Am J Clin Pathol 94:554–560.

Chang DY, Chen RJ, Lee SC, Huang SC. 1997. Prevalence of single and multiple infection with human papillomaviruses in various grades of cervical neoplasia. J Med Microbiol 46:54–60.

De Villiers E-M. 1994. Human pathogenic papillomavirus types: an update. In: zur Hausen H, editor. Human papillomaviruses. Heidelberg, Germany: Springer-Verlag. p 1–12.

Gravitt P, Hakenewerth A, Stoerker J. 1991. A direct comparison of methods proposed for use in widespread screening of human papillomavirus infections. Mol Cell Probes 5:65–72.

Herrington CS. 1994. Human papillomaviruses and cervical neoplasia. I. Classification, virology, pathology, and epidemiology. J Clin Pathol 47:1066–1072.

Herrington CS. 1995. Human papillomaviruses and cervical neoplasia. II. Interaction of HPV with other factors. J Clin Pathol 48:1–6.

Hong Kong Cancer Registry, editor. 1992. Cancer incidence and mortality in Hong Kong. Hong Kong: Hospital Authority. p 20.

Huang S, Afonina I, Miller BA, Beckmann AM. 1997. Human papillomavirus types 52 and 58 are prevalent in cervical cancers from Chinese women. Int J Cancer 70:408–411.

Kwok S, Higuchi R. 1989. Avoiding false positives with PCR. Nature (Lond) 339:237–238.

Lauricella-Lefebvre M-A, Piette J, Lifrange E, Lambotte R, Gerard P, Rentier B. 1992. High rate of multiple genital HPV infections detected by DNA hybridization. J Med Virol 36:265–270.

Liaw K-L, Hsing AW, Chen C-J, Schiffman MH, Zhang TY, Hsieh C-Y, Greer CE, You S-L, Huang TW, Wu T-C, Leary TJ, Seidman JD, Blot WJ, Meinert CL, Manos MM. 1995. Human papillomavirus and cervical neoplasia: a case control study in Taiwan. Int J Cancer 62:565–571.

Lorincz AT, Reid R, Jenson AB, Greenberg MD, Lancaster W, Kurman RJ. 1992. Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. Obstet Gynecol 79:328–337.

Lungu O, Sun XW, Felix J, Richart RM, Silverstein S, Wright TC. 1992. Relationship of human papillomavirus type to grade of cervical intraepithelial neoplasia. JAMA 267:2493–2496.

Matsukura T, Sugase M. 1990. Molecular cloning of a novel human papillomavirus (type 58) from an invasive cervical carcinoma. Virology 177:833–836.

Munoz N, Bosch FX. 1992. HPV and cervical neoplasia: review of case-control and cohort studies. In: Munoz N, Bosch FK, Shah KV, Meheus A, editors. The epidemiology of human papillomavirus and cervical cancer. Publication no. 119. Lyon, France: International Agency for Research on Cancer. p 251–261.

Pao CC, Kao S-M, Tang G-C, Lee K, Si J, Ruan S. 1993. Human papillomavirus and cervical carcinoma in China and Taiwan. Lancet 342:937.

Pao CC, Kao S-M, Tang G-C, Lee K, Si J, Ruan S. 1994. Prevalence of human papillomavirus DNA sequences in an area with very high incidence of cervical carcinoma. Br J Cancer 70:694–696.

Saiki RA, Bugawan TL, Horn GT, Mullis KB, Erlich HA. 1986. Analysis of enzymatically amplified β-globin and HLA DQ alpha DNA with allele specific oligonucleotide probes. Nature (Lond) 324:163–166.

Ting Y, Manos MM. 1990. Detection and typing of genital human papillomavirus. In: Innis MA, Gelfond DH, Sninsky JJ, White TJ, editors. PCR protocol: a guide to methods and applications. San Diego, CA: Academic Press. p 356–367.

Vandenvelde C, Scheen R, Van Pachterbeke C, Loriaux C, Decelle J, Hubert T, Delhaye C, Cattoor J-P, Duys M, Van Beers D. 1992. Prevalence of high risk genital papillomaviruses in the Belgian female population determined by fast multiplex polymerase chain reaction. J Med Virol 36:279–282.

Van Ranst M, Tachezy R, Burk RD. 1996. Human papillomaviruses: a never-ending story? In: Lacey C, editor. Papillomavirus reviews: current research on papillomaviruses. Leeds, UK: Leeds University Press. p 1–20.